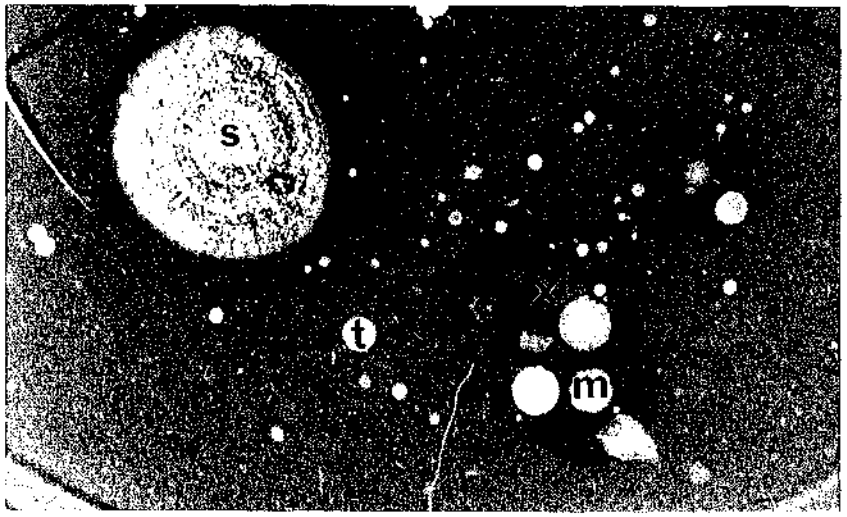

PLATE 3. A river water sample plated on to chitin-actidione agar. Growing colonies of *Streptomyces* (s) and *Micromonospora* (m) show hydrolysis zones, as also do myxobacteria (x). Other bacteria (t) are growing but not producing hydrolysis zones. (×3).

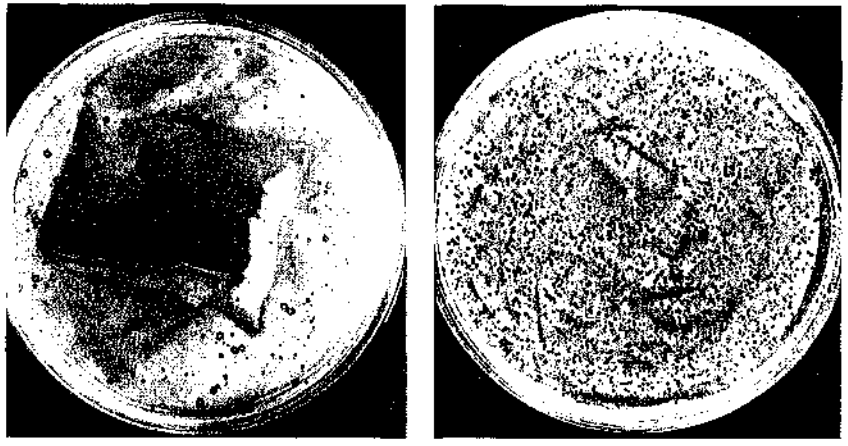

PLATE 4. Plaque formation in *Actinoplanes missouriense* (left) and in *Streptomyces griseus* (right) following phage recovery from local oak leaf litter and stream water respectively. (×0·6).

ACTINOMYCETES AND ACTINOPHAGE IN FRESH WATER

L. G. WILLOUGHBY

The Actinomycetales, commonly referred to as actinomycetes, are a group of micro-organisms which have a true branching mycelium and so resemble the fungi, but are small in size and have other characters more like those typical of bacteria. They thus form a group lying in classification half-way between the fungi and the bacteria. If the mycelium is short lived in sub-culture, or even becomes absent altogether, it is difficult to decide whether the strain should be called an actinomycete or a bacterium. Strains now included in the genera *Nocardia* and *Mycobacterium*, or more loosely placed within this taxonomic area (e.g. Nocardioform-Lspi, discussed below) frequently give rise to such a dilemma. Again, actinomycetes resemble fungi because they may release motile spores from sporangia, but these spores are as small (about 1 μm diameter) as motile bacteria.

Several other rather fundamental characters of actinomycetes are those of procaryotic cells (such as those of bacteria and blue-green algae) rather than those of eucaryotic cells (as found in fungi, green algae and higher plants); procaryotic characters are their lack of membrane-bounded organelles such as nuclei and mitochondria, their frequent susceptibility to phage-virus and their type of antibiotic sensitivity. They are killed by many of the antibiotics which also kill bacteria.

Records of direct observations made on aquatic actinomycetes growing in their natural milieu are virtually non-existent so far, although I did find a few sporangia of *Actinoplanes missouriensis* Couch on twigs left exposed in a stream for several weeks, after I had been encouraged to search by a high level of recovery of this species in agar-plated washings from parallel exposures. Considering more indirect procedures, the technique of damp incubation has potential, and by using this on damp allochthonous leaf material collected on the shore of Windermere I was able to observe the development of a variety of sporangial forms of the family Actinoplanaceae. Although *Actinoplanes* was the dominant form recovered, strains of *Spirillospora* and of other unidentified genera were also present (Willoughby, 1969).

In isolating actinomycetes from nature on to nutrient growth media, addition of actidione (cycloheximide) eliminates fungi but unfortunately there are no antibiotics which will satisfactorily segregate growing actinomycetes and bacteria as two distinct groups. However, in making general surveys of actinomycetes in fresh water, it is probably better to allow the full spectrum of procaryotic cells (both bacterial and actinomycete) to develop on the initial isolation medium rather than to attempt such a segregation. Where a single actinomycete only is searched for, the use of a more selective method becomes a possibility. Thus Cross & Attwell (1974) have employed the antibiotic novobiocin (in conjunction

with the usual actidione addition) and an elevated incubation temperature to gain almost pure growths of *Thermoactinomyces* on the first isolation dishes prepared from the benthic mud of Thirlmere and Windermere.

In my general actinomycete surveys the plating of leaf washings, water samples and mud dilutions on to nutrient agar (with incorporated actidione) has been very productive. The nutrient agar used in this work contained only colloidal chitin (the sole carbon and nitrogen source) and some mineral salts (Plate 3). I chose chitin as the organic growth source because it is known that virtually all actinomycetes, but few bacteria, possess the enzymes to digest it. It might be expected that this would mean that actinomycetes would be found associated with chitin in nature, but this does not appear to be so; neither the chitinous exo-skeletons of Crustacea nor the case skins of insects are obviously being decayed by aquatic actinomycetes. This failure to reconcile a laboratory finding with a field situation is not unknown for other micro-organisms.

The agar plating of washings from damp oak leaves collected from the shore of Windermere has proved particularly productive of actinomycetes. In addition to the Actinoplanaceae (e.g. *Actinoplanes missouriensis*) which formed sporangia directly on the agar, others remained sterile but could be induced to fruit in sub-culture on a minimal growth medium which incorporated purified humic acids (Willoughby, Baker & Foster 1968). This fruiting effect was investigated further using chemically defined fractions of the humic acids (Willoughby & Baker 1969). Other actinomycetes from the leaf washings appear to be new to science and are characterized by the possession of motile spores (with polar groups of 2-5 flagella) borne on phialides which are miniature editions of those produced by many aquatic fungi (Willoughby 1969). Later work has suggested that the Actinoplanaceae are even more active as agents of decay in streams, where they grow on wood as well as on leaves (Willoughby 1971).

Actinomycetes recovered from surface benthic muds of local lakes (including ones in use as reservoirs) are predominantly *Micromonospora* and *Streptomyces* strains, similar to those which occur in soils of the drainage basins. Do these benthic mud recoveries relate to active populations or can they be attributed solely to the wash-in of viable spores? From ingenious laboratory experiments involving differential cell-killing in a high speed blender, and using Windermere mud, Johnston (1972) concluded that *Micromonospora* probably was active but left the issue open in regard to *Streptomyces*. This is the genus which is notorious for odour production. The odour, due apparently to complex aliphatic alcohols, can be troublesome in a domestic water supply, especially because it largely survives treatment by chlorine, ozone or permanganate. Adsorption on to activated carbon will eliminate it, however. Because it is known that the *Streptomyces* content of benthic mud is a reflection of the productive status of a lake (for example, it is greater in Blelham Tarn than in Wastwater) and as there are prospects that lowland, more

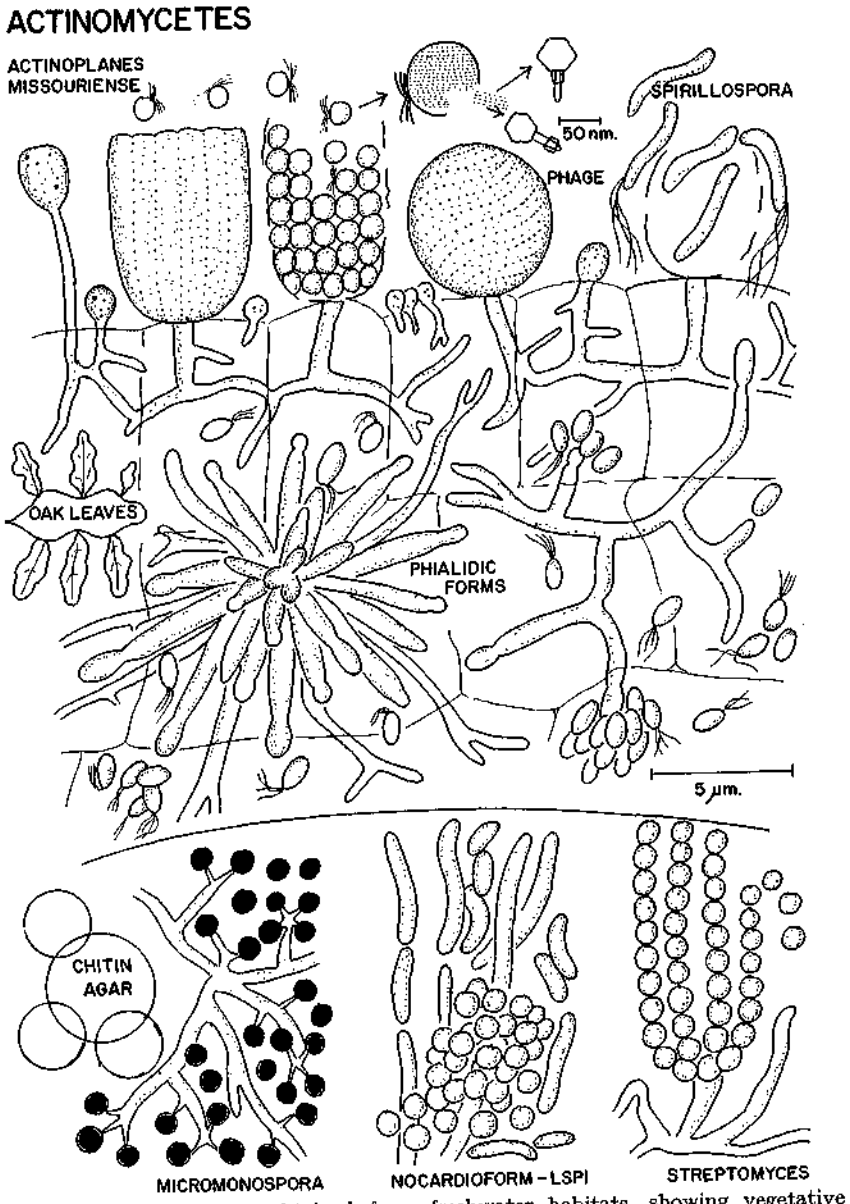

FIG. 1. Actinomycetes obtained from freshwater habitats, showing vegetative growth and release of reproductive spores.

productive, waters may be used for domestic supplies, this subject of odour production is of contemporary interest. I have attempted to cast light on the possible activity of *Streptomyces* in benthic lake mud by studying the *Streptomyces*-phage; this is discussed below.

Actinophage

Phages are virus particles which, on liberation from the host cells which harboured them, show a definite morphological structure with a polyhedral head and an attached tail of varying length. Virulent phages replicate inside the host cells and destroy them completely by lysis, but other phages are known which, although they also may replicate internally, normally have no obviously deleterious effect on the host cells. Host cells carrying such phages, which may be provoked into lytic activity by chemical or biological stimulus, are said to be lysogenic. Both virulent phages (actinophages) and lysogenic host strains are reported for the actinomycetes.

Polyvalency in virulent actinophages occurs when they can be transmitted from one host strain to another host strain within one species or from one host species to another within one genus. I have never achieved inter-generic transmission with actinophage, although I have attempted it. I have made a study of the distribution of virulent phages of local actinomycetes in the hope that it will yield ecological information about the hosts. The rationale for this study is as follows. Virulent phage attacks host material which is growing actively, and it is therefore likely to occur in quantity in situations where this condition applies. On the other hand, in situations where the host is absent, or is present but inactive, there is a lower expectation of occurrence of its phage. The co-existence in one locality of a virulent actinophage and a host which is subsceptible to it (reported below) would appear to be anomalous. It might be expected that such a situation would lead to the elimination of a sensitive portion of the actinomycete host population and its replacement by phage-resistant mutants or lysogenic strains. On this basis, selection pressure by actinophage on the hosts would be very great. However, this argument pre-supposes that the actinophage has uninhibited contact with the host cells in nature, and laboratory experiments with bacteriophage have suggested (Anderson 1957) that extremely high concentrations of phage are necessary to ensure this. Although I have not considered in detail actinophage concentration in the natural environment, the general pattern of my results suggests that selection pressure on potential hosts is not unduly high. I have searched for actinophage, using the technique of host enrichment in peptone-yeast extract broth, with simultaneous exposure to water, mud or soil collections. After incubation a purified extract of the enrichment has been tested for activity, as demonstrated by plaque formation (areas of lysis) in the particular host under examination (Plate 4).

Knowing that *Actinoplanes* was almost certainly active in the damp allochthonous oak leaf litter collected on the Windermere shore line, here was the obvious place to begin a search for its phage. This was found immediately, and under the electron microscope it had a particular type of fine structure which is, so far, undescribed for any other actinophage (Willoughby, Smith & Bradshaw 1972). In particular it had a conspicuous tail sheath which was presumed to be contractile, since it was found in different dispositions in relation to the tail itself.

In descending order of natural concentration its distribution pattern was as follows: a high concentration in the littoral allochthonous leaves of local lakes, a fairly high concentration in local stream water feeding these lakes (for example, one viable unit per ml in Wilfin Beck), a lower concentration in the local lake water itself (one viable unit per 25 ml in Blelham Tarn and only one viable unit per 100 ml in Windermere), and an undetectable concentration in the benthic mud of these lakes. Thus the pattern of phage distribution follows the presumptive pattern of host activity, which is that it occurs on lake shores in the cast-up leaves; it occurs in local streams where the substratum is submerged leaves and wood and the fragments derived from these; it does not occur in benthic lake mud. Wash-in of *Actinoplanes* phage in a viable condition into the lake deposits might perhaps have been expected, even in the absence of its host, but apparently this did not take place.

Nocardioform-Lspi was first reported from Blelham Tarn (Willoughby 1969a) but is now known to have a more widespread distribution, at least in the north of England. In the absence of any contradictory evidence it was described then as a novel aquatic actinomycete, occurring in the lake inflows, the lake water, and in the benthic mud beneath. Although repeated searches were made for it, actinophage for this actinomycete was never recovered from these situations. A recent development of great interest is that Cross & Rowbotham (1974 and personal communications) have shown that Nocardioform-Lspi is in fact not aquatic but grows on the dung of herbivorous animals, such as sheep, in the pasture fields of the drainage basin. Furthermore, they have recovered the specific phage from the herbivorous-animal dung also. Here we see an interesting vindication of the phage-location method as a clue to host activity and the suggestion that washed-in phage may retain its viability less persistently than the washed-in host. This does not accord with the mythological view that viruses, being more of a chemical than a biological nature, are potentially immortal.

A study of the aquatic phages of *Streptomyces* (Willoughby, 1976) was greatly complicated by the local multiplicity of host species, by obvious wash-in from the soil, and by the high degree of polyvalency which the phages exhibit. However, by deduction it has been concluded that certain pink-spored *Streptomyces* strains are part of the indigenous and active aquatic microflora of the benthic mud in Blelham Tarn. In this study the opportunity was taken to expose an authentic soil strain of

Streptomyces griseus, isolated outside the Lake District, to local phage. Phage activity was never demonstrated from local soils but, most unexpectedly it was demonstrated from local stream water. I considered the possibility that the enrichment solutions derived from the soils contained colloids or cations which were deleterious to phage adsorption on to *S. griseus*, while the enrichment solutions derived from stream water were chemically cleaner and readily allowed phage adsorption. However, this possibility had to be rejected. An explanation for the observations might be that washed-in *Streptomyces* phage from soil remains viable and somehow changes its polyvalency range in water. It can then lyse a new host, which was immune in the terrestrial environment.

This is an appropriate place to acknowledge the contributions to our knowledge of Lake District actinomycetes made by Dr T. Cross and his students at Bradford University. I have drawn on some of these in my article. In my own work I have been greatly assisted by Mrs P. J. McDougall and Mrs S. M. Smith.

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THE COMMUNITIES OF RUNNING WATER IN CUMBRIA

T. T. Macan

Early Work

Important studies of the communities in streams and rivers were made between the wars by F. T. K. Pentelow, R. W. Butcher and other members of the Ministry of Agriculture's team, and by E. Percival and H. Whitehead operating from Leeds. They were, however, handicapped by inadequate keys to the immature stages of Plecoptera, Ephemeroptera, Trichoptera and Diptera (notably Chironomidae), four groups whose larvae constitute a large proportion of the fauna of unproductive rapidly flowing water.

In 1937 H. B. N. Hynes arrived at Wray Castle as a research student and started work which resulted in a long paper on the taxonomy of nymphal Plecoptera (1941) and later a key in the *Scientific Publications* series (1967)*. This was followed by a similar key for the Ephemeroptera (Macan 1970).* When nymphs could be identified, work on life history and migration (Macan 1957a) started in Ford Wood Beck, a stony stream running through agricultural land. A comparison with other streams (1957b) followed, and Jean Mackereth, who was my assistant at the time, undertook a study of the Plecoptera (1957) and Trichoptera (1960). She also described and distinguished the larvae in two groups of caddis (1954, 1956). T. Gledhill succeeded Mrs Mackereth and studied the water mites (1971). He also climbed Helvellyn once a week to study the life history of the mayfly *Ameletus inopinatus* (1959) and to compare the emergence periods of various species in Whelpside Ghyll at 2000 ft and in Ford Wood Beck (1960). A further study of captures in emergence traps (Macan 1964) and a study of the amphipod *Gammarus* (Macan & Mackereth 1957) gave a comprehensive picture of the fauna of Ford Wood Beck.

There were 9 species of Ephemeroptera and 14 species of Plecoptera but the former were by far the more numerous. *Gammarus* was very abundant and maintained remarkably constant numbers, *Ancylus* (Mollusca) was numerous in places and a third characteristic species was *Agapetus fuscipes* (Trichoptera).

After the survey was complete the stream was enriched by effluent from an overloaded septic tank, which led to an enormous increase in the numbers of *Polycelis felina*. A simultaneous decrease in the number of some other species was probably due to predation by this flatworm (Macan 1962).

Temperature was recorded for several years (Macan 1958) and the effect of temperature on two species was observed (Macan 1960a, b).

* These are the dates of the most recent editions.